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Isolation and Identification of Pathogenic Microorganisms at Wastewater-Irrigated Fields: Ratios in Air and Wastewater

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Samples of air and corresponding wastewater samples were taken at wastewater spray-irrigated fields. The concentrations of salmonellae and enteroviruses present in these samples were determined and compared with those of coliforms, and the ratios between them were calculated. The most common *Salmonella* serotype in the air was *Salmonella ohio*, whereas in the wastewater, *Salmonella anatum* was the most common. Enteroviruses isolated and identified were poliovirus, echovirus, and coxsackievirus type B. From the ratios of salmonellae to coliforms and enteroviruses to coliforms in the air, as compared to these ratios in the wastewater, it was concluded that the suitability of coliforms as an indication of airborne contamination caused by spray irrigation is questionable.

The treatment of wastewater by activated sludge, trickling filters, and spray irrigation of land may release to the air certain pathogenic organisms (12, 29) that have survived these treatments (7). The airborne transmission of the pathogenic organisms is of growing concern (13, 30), and some presumptive evidence indicates that increased enteric disease incidence among nearby residents may be associated with such wastewater aerosols (14).

The enteric bacteria are perhaps the most common pathogens present in wastewater, and of these, *Salmonella* species occur most frequently. These microorganisms maintain their viability in wastewater, and it has been reported (2) that *Salmonella* densities of 5,000 per liter may be found in raw sewage. A *Salmonella* bacterium was isolated from air 60 m downwind from a sprinkler wastewater irrigation line (16). It has also been reported that the ratio of aerosolized salmonellae to coliforms near a sewage treatment plant was identical to that in the wastewater (11). It must be mentioned that the diseases normally regarded as airborne are not the only source of airborne infection, and primates may be infected with *Salmonella typhi* through inhalation of a dose that is 1,000 times lower than the normal infective dose (6).

Approximately 100 types of enteric viruses may be present in raw sewage. Viruses found in concentrations of several thousand viral units per liter can generally be isolated from sewage with currently available isolation procedures. Berg (3) has reviewed the literature and found that reported virus removals by secondary sewage treatment vary from 0 to 99%. Although it has been shown that certain viruses can travel very long distances under varied weather conditions and may initiate infection at points far from their sources (31), a literature review indicates that the potential health hazards to populations exposed to airborne viruses at spray and aeration sites have not been successfully evaluated. Only in two reports (13, 30) is a successful sampling of enteric viruses from wastewater aerosols mentioned.

Coliform bacteria have served as indicators of fecal contamination of water for many years, and their densities have been utilized as criteria for the degree of pollution. Some investigators have suggested that these organisms would serve equally well as indicators of airborne contamination by wastewater treatment plants (1, 10). Other investigators emphasized the poor survival of coliform bacteria in the airborne state relative to other airborne microorganisms and suggested the possibility of using *Klebsiella* (21, 22, 28), *Alcaligenes faecalis* (18), *Streptococcus faecalis* (5), and coliphages (8) as indicator organisms in the air. The disadvantage of these suggested organisms is that the procedures for their differentiation are generally more involved than those required for coliform determinations. Since coliform organisms are universally accepted as indicators of water contamination, they were examined as possible indicators of air contamination from wastewater irrigation fields. The purpose of this study is to determine the occurrence of *Salmonella* bacteria and animal enteroviruses in aerosol emissions from wastewater irrigation sites by direct assay and to evaluate and compare the suitability of coliforms as indicators of airborne contaminations.

MATERIALS AND METHODS

Sampling location. The air in the vicinity of an effluent-irrigated field located near Kibbutz Tzora's (a collective settlement) was sampled. The sampling site and its effluent were described previously (16). The sprinklers used were Na'an types no. 233/92, 254/91, and 2812 (Na'an Metal Works, Israel) with discharges of 1.7, 4.5, and 100 m^3 h^{-1}, respectively. The last one was operated as a single sprinkler. All three sprinkler types operated at identical water pressure conditions.

Air sampling. Air samples (27 ± 11 m^3 of air) for *Salmonella*, total coliform, and enterovirus assays were taken at different distances downwind from a wastewater irrigation line with a large-volume scrubber-cyclone type sampler (4). The operation conditions and the collecting fluid were as described previously (30), except that the collecting fluid in the second sampling period (summer 1978) was changed to 1% beef extract (Oxoid). The samples were kept on ice during transport to the laboratory, where they were assayed on the same day for bacteria determination or kept at −80°C for virus determination.

Microbial methods. (i) Coliforms. Total coliform levels in the sampler collecting fluid and in the wastewater were estimated by M-endo broth (Difco) with 15% agar (Difco) in plastic petri dishes. The inoculated dishes were incubated for 24 h at 37°C. Results are reported as colony-forming units (CFU) per 100 ml of wastewater or cubic meter of air.

(ii) Salmonella. The most-probable-number (MPN) method was used for estimating quantitatively the levels of *Salmonella* in the sampler collecting fluid and in the wastewater. Sample volumes of 10.0, 1.0, and 0.1 ml of the collecting fluid, and an additional volume of 0.01 for the wastewater, were inoculated into appropriate five-tube replications of selenite broth (Difco). The tubes were incubated at 42°C for 48 h, after which they were streaked onto brilliant green agar (Oxoid) and salmonella-shigella agar (Difco) plates. The inoculated plates were then incubated at 37°C for 24 h. Colonies presumed to be *Salmonella* were isolated and screened on Kligler iron agar (Difco) slants, and suspected *Salmonella* cultures were confirmed with polyvalent O antiserum by slide agglutination. Final identification was performed at the National Salmonella Center, Government Central Laboratories, Ministry of Health, Jerusalem, Israel. Results are reported as *Salmonella* MPN per 100 ml of wastewater or *Salmonella* MPN per cubic meter of air.

(iii) Viruses. The airborne virus determination method in the first sampling period (summer 1977) has been described previously (30). In the second sampling period (summer 1978), the following procedure was carried out. The whole collecting fluid of each sample was mixed with an equal volume of minimum essential medium, double strength, containing 4% fetal bovine serum and 20 ml of antibiotic solution per liter, which contained 2×10^5 U of penicillin ml^{-1}, 200 mg of streptomycin ml^{-1}, 200 mg of kanamycin ml^{-1}, 25 mg of neomycin ml^{-1}, and 10^5 U of mycostatin ml^{-1}. From this mixture, 2-ml samples were inoculated to test tubes of buffalo green monkey (BGM) cell cultures. Tubes were capped, incubated at 37°C for as long as the integrity of the cell sheet permitted, and observed for cytopathic effect 7 to 14 days after inoculation. Samples of medium from each tube that showed cytopathic effect were subinoculated twice into other culture tubes for confirmation of positive virus effect. Positive culture tubes were thereafter considered as virus isolates. Typing of these isolates was done by the Lim Benyesh-Melnick neutralization method (20). The results are expressed as enterovirus isolates per cubic meter of air.

Liquid sewage samples of 2 liters were concentrated by the adsorption-elution (23) and organic flocculation methods (15) and then assayed for polioviruses by the fluorescent-antibody assay (17) and for total enteroviruses by the plaque method on BGM cells (26). The results are reported as plaque-forming units per liter.

RESULTS

Throughout this study different types of sprinklers were used, and consequently different discharge rates, particle numbers, and aerosol volumes were generated in the experimental field (Tables 1 and 2). The frequency of positive *Salmonella* samples was found to be 78% in the wastewater and 18% in the airborne emissions. For enteroviruses, the frequency of positive samples was 71% in wastewater and 44% in aerosol emissions, indicating higher frequency of enteroviruses in aerosol emissions in comparison to *Salmonella*.

Total coliforms and *Salmonella* densities in wastewater sprayed by sprinklers, and the ratios between them, are represented in chronological order in Table 3. Ratios of *Salmonella* to total coliforms of $0.14{:}10^6$ to $10.0{:}10^6$ were originated by 2 to 60 MPN of *Salmonella* per 100 ml and total coliform levels of 1.5×10^6 to 1.4×10^7 CFU per 100 ml. Table 4 represents the densities and ratios for enteroviruses. Ratios of enteroviruses to total coliforms of $0.03{:}10^6$ to $809.0{:}10^6$ were originated by 30 to 8.9×10^4 plaque-forming units of enterovirus per liter and total coliform levels of 1.0×10^6 to 1.5×10^8 CFU per 100 ml. Table 1 represents the total coliform and *Salmonella* densities and the ratios between them in the aerosol emissions. Sampling distances varied from 40 to 200 m from the irrigation line. All of the positive *Salmonella* air samples were from a distance of 40 m, except one from 100 m when a sprinkler with a very large discharge was used. Ratios of salmonellae to total coliforms of $4.9{:}10^6$ to $1{,}046{:}10^6$ were originated by *Salmonella* levels of 3.2×10^{-2} to 5.4×10^{-2} MPN m^{-3} of air and by total coliform levels of 43 to 1,076 CFU m^{-3} of air. In air sampling for enteroviruses (Table 2), the distance from the source varied from 36 to 100 m, and positive air samples were found throughout this range. Ratios of enteroviruses to total coliforms of $136{:}10^6$ to $6{,}583{:}10^6$ were originated by enterovirus levels of 2.5×10^{-2} to 1.4×10^{-1} isolates m^{-3} and by total coliform levels of 11 to 603 CFU m^{-3}.

TABLE 1. *Total coliform and Salmonella levels in airborne emissions*

Date of sampling	Distance from source (m)	Sprinkler type[a]	Total coliforms (CFU m^{-3})	Salmonellae (MPN m^3)	Salmonellae/total coliforms
25 May 1977	40	233/92	177	0	0
			413	0	0
5 June 1977	40	254/91	591	0	0
			18	0	0
20 June 1977	40	254/91	347	0	0
			358		
29 June 1977	40	233/92	126	0	0
			212	0	0
11 July 1977	40	233/92	44	0	0
			82	0	0
			62	0	0
16 Aug. 1977	40	254/91	43	4.5×10^{-2}	$1,046:10^6$
28 May 1978	40	233/92	253	0	0
4 June 1978	40	254/91	38	0	0
4 July 1978	60	254/91	34	0	0
9 July 1978	60	233/92	43	0	0
11 July 1978	40	233/92	144	4.8×10^{-2}	$333:10^6$
18 July 1978	40	233/92	1,076	5.3×10^{-2}	$49:10^6$
			859	5.4×10^{-2}	$63:10^6$
25 July 1978	60	233/92	75	0	0
			47	0	0
1 Aug. 1978	60	233/92	235	0	0
9 Aug. 1978	60	254/91	533	0	0
			254	0	0
15 Aug. 1978	40	233/92	916	5.2×10^{-2}	$58:10^6$
			631	0	0
31 Aug. 1978	200	254/91	26	0	0
22 Sept. 1978	120	2812	501	0	0

[a] Discharges of sprinkler types no. 233/92, 254/91, and 2812 are 1.7, 4.5, and 100 m^3 h^{-1}, respectively.

TABLE 2. *Total coliform and enterovirus levels in airborne emissions*

Date of sampling	Distance from source (m)	Sprinkler type[a]	Total coliforms (CFU m^{-3})	Total enteroviruses (BGM cells) (isolates m^{-3})	Enteroviruses/total coliforms
8 June 1977	40	233/92	219	0	0
	40		78	0	0
	50		207	1.4×10^{-1}	$676:10^6$
19 July 1977	42	254/91	84	0	0
28 Aug. 1977	36	254/91	23	0	0
1 Sept. 1977	40	254/91	603	8.2×10^{-2}	$136:10^6$
12 Sept. 1977	40	254/91	82	2.5×10^{-2}	$305:10^6$
16 Sept. 1977	70	254/91	30	2.6×10^{-2}	$867:10^6$
20 Sept. 1977	70	254/91	5	0	0
21 Sept. 1977	100	254/91	11	4.8×10^{-2}	$4,364:10^6$
25 Sept. 1977	100	254/91	12	0	0
10 Oct. 1977	−70	254/91	0	0	0
27 July 1978	40	233/92	182	0	0
8 Aug. 1978	100	233/92	12	0	0
			12	7.9×10^{-2}	$6,583:10^6$
16 Aug. 1978	100	254/91	292	0	0
17 Aug. 1978	100	254/91	191	1.0×10^{-1}	$524:10^6$

[a] Discharges of sprinkler types no. 233/92 and 254/91 are 1.7 and 4.5 m^3 h^{-1}, respectively.

The ratio of enteroviruses to total coliforms increased by about one log as the sampling distance increased from the mean initial distance of 43 m to a distance of 100 m (Fig. 1).

Table 5 summarizes results of 28 samplings of wastewater and air which were checked for salmonellae and coliforms. The table includes the arithmetic means and standard deviations for the *Salmonella* and coliform densities in wastewater; the ratio of salmonellae to coliforms in wastewater; the *Salmonella* and coliform densities in the air; the ratio of salmonellae to coliforms in the air; the density of salmonellae in the air normalized with respect to their density in wastewater; and the density of coliforms in the air normalized with respect to their density in wastewater. All of these variables were arranged in the table according to rising densities of salmonellae in the wastewater.

Correlation coefficients were determined between 28 values of *Salmonella* densities in wastewater and the rest of the variables calculated in these samplings, respectively. Good correlation coefficients were found for the ratio of salmonellae to coliforms in wastewater (0.75), the *Salmonella* density in the air (0.70), and the ratio of salmonellae to coliforms in the air (0.70). When the correlation coefficients were calculated between the density of salmonellae in the air and the rest of the variables, respectively, a good correlation was found for the normalized density of salmonellae in the air (0.77). In addition, it was found that the general mean of the ratio of salmonellae to coliforms in the air was 26.7 times higher than the general mean of the ratio of salmonellae to coliforms in wastewater. The mean density of salmonellae in the air normalized with respect to their density in the wastewater was fivefold higher than the mean density of coliforms normalized with respect to their density in the wastewater; with the aid of the Wilcoxon matched-pairs signed-ranks test

TABLE 3. *Total coliform and Salmonella levels in wastewater*

Date of Sampling	Total coliforms (CFU/100 ml)	Salmonellae (MPN/100 ml)	Salmonellae/total coliforms
25 May 1977	7.0×10^6	4	0.57:10^6
5 June 1977	2.4×10^6	10	4.17:10^6
20 June 1977	1.4×10^7	2	0.14:10^6
29 June 1977	1.0×10^7	0	0
11 July 1977	1.0×10^7	20	2.0:10^6
16 Aug. 1977	1.3×10^7	60	4.62:10^6
28 May 1978	6.4×10^6	0	0
4 June 1978	1.3×10^7	4	0.31:10^6
4 July 1978	8.5×10^6	8	0.94:10^6
9 July 1978	4.0×10^6	0	0
11 July 1978	5.0×10^6	8	1.60:10^6
18 July 1978	3.3×10^6	33	10.00:10^6
25 July 1978	3.5×10^6	2	0.57:10^6
1 Aug. 1978	3.6×10^6	5	1.39:10^6
9 Aug. 1978	1.5×10^6	0	0
15 Aug. 1978	3.5×10^6	21	6.00:10^6
31 Aug. 1978	8.6×10^6	2	0.23:10^6
22 Sept. 1978	1.8×10^6	2	1.11:10^6

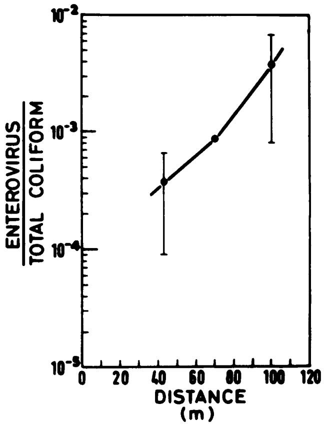

FIG. 1. *Ratio of airborne enteroviruses to total coliforms versus sampling distance.*

TABLE 4. *Total coliform and enterovirus levels in wastewater*

Date of sampling	Total coliforms (CFU per liter)	Total enteroviruses (BGM cells) (plaque-forming units per liter)	Polioviruses (FA method[a]) (plaque-forming units per liter)	Enteroviruses/total coliforms
8 June 1977	1.0×10^8	6.5×10^2	3.7×10^2	6.50:10^6
19 July 1977	3.1×10^7	1.1×10^1	6.9×10^1	2.23:10^6
28 Aug. 1977	1.6×10^8	6.0×10^0	1.2×10^2	0.75:10^6
1 Sept. 1977	1.5×10^9	5.2×10^1	0	0.03:10^6
12 Sept. 1977	3.8×10^7	3.0×10^1	0	0.79:10^6
16 Sept. 1977	1.0×10^7	1.7×10^2	0	17.00:10^6
20 Sept. 1977	1.7×10^8	1.3×10^4	1.1×10^4	76.47:10^6
21 Sept. 1977	3.0×10^8	3.3×10^2	1.8×10^2	1.10:10^6
25 Sept. 1977	1.1×10^8	8.2×10^4	8.9×10^4	809.09:10^6
10 Oct. 1977	2.2×10^8	3.2×10^2	0	1.45:10^6
27 July 1978	1.2×10^8	0	0	0
8 Aug. 1978	2.4×10^7	0	0	0
16 Aug. 1978	5.3×10^7	0	0	0
17 Aug. 1978	3.7×10^7	0	0	0

[a] Reference 24.

(27), it was found that the difference in these ratios is not significant.

Table 6 represents 16 samplings of wastewater and air which were checked for enteric viruses and coliforms. The table shows the arithmetic mean and standard deviation of the same variables expressed in Table 5, and their values were arranged according to increased values of virus densities in wastewater. A high correlation coefficient (0.99) was found only between the density of enteroviruses in the wastewater and the ratio of enteroviruses to coliforms in the wastewater. The mean ratio of enteroviruses to coliforms in wastewater was 14.5-fold higher than the mean of that same ratio in the air. On the other hand, the mean enterovirus density in the air normalized with respect to the density in the wastewater was 123-fold higher than the mean normalized density of coliforms. These differences were also found to be not significant according to the

TABLE 5. *Mean concentrations and ratios of total coliforms to Salmonella in wastewater and airborne emissions*[a]

Quartile	Wastewater			Air			Salmonella: air/wastewater	Total coliforms: air/wastewater
	Salmonellae (MPN/100 ml)	Total coliforms (CFU/100 ml)	Salmonellae/total coliforms	Salmonellae (MPN m^{-3})	Total coliforms (CFU m^{-3})	Salmonellae/total coliforms		
1st	0.3	5.1×10^6	1.6×10^{-7}	0	274	0	0	1.3×10^{-4}
	(0.8)	(3.8×10^6)	(4.2×10^{-7})		(182)			(1.4×10^{-4})
2nd	2.6	8.2×10^6	4.1×10^{-7}	0	206	0	0	2.4×10^{-5}
	(1.0)	(4.4×10^6)	(2.1×10^{-7})		(164)			(1.7×10^{-5})
3rd	9.3	6.4×10^6	2.1×10^{-6}	6.9×10^{-3}	158	4.8×10^{-5}	8.6×10^{-4}	5.2×10^{-6}
	(5.2)	(4.1×10^6)	(1.5×10^{-6})	(1.8×10^{-2})	(207)	(1.2×10^{-4})	(2.3×10^{-3})	(9.0×10^{-5})
4th	29.7	6.6×10^6	5.8×10^{-6}	2.9×10^{-2}	524	1.7×10^{-4}	9.2×10^{-4}	1.5×10^{-4}
	(14.6)	(4.2×10^6)	(3.3×10^{-6})	(2.8×10^{-2})	(451)	(3.7×10^{-4})	(1.0×10^{-3})	(1.4×10^{-4})
Total	10.5	6.6×10^6	2.1×10^{-6}	9.0×10^{-3}	291	5.4×10^{-5}	4.4×10^{-4}	8.8×10^{-5}
	(13.9)	(4.1×10^6)	(2.9×10^{-6})	(2.0×10^{-3})	(298)	(2.0×10^{-4})	(1.2×10^{-3})	(1.2×10^{-4})
Coefficient of correlation:[b]								
Wastewater[b]		0.12	0.75	0.68	0.28	0.70	0.26	0.18
Air[c]					0.55	0.54	0.77	0.39

[a] Mean ± standard deviation (in parentheses).

[b] Coefficients of correlation (r) between *Salmonella* concentration in wastewater or air, as indicated and other parameters ($n = 28$).

TABLE 6. *Mean concentrations and ratios of total coliforms and enteric viruses in wastewater and airborne emissions*[a]

Quartile	Wastewater			Air			Enteroviruses: air/wastewater	Total coliforms: air/wastewater
	Enteroviruses (PFU/100 ml)	Total coliforms (CFU/100 ml)	Enteroviruses/total coliforms	Enteroviruses (per m^3)	Total coliforms (CFU m^{-3})	Enteroviruses/total coliforms		
1st	0	3.5×10^6	0	4.5×10^{-2}	127	1.8×10^{-3}		2.9×10^{-5}
		(1.4×10^6)		(5.2×10^{-2})	(139)	(3.2×10^{-3})		(2.8×10^{-5})
2nd	1.2	8.7×10^6	9.4×10^{-7}	6.3×10^{-3}	93	7.6×10^{-5}	2.1×10^{-3}	1.6×10^{-5}
	(1.3)	(6.3×10^6)	(9.3×10^{-7})	(1.2×10^{-2})	(66)	(1.5×10^{-4})	(4.1×10^{-3})	(1.1×10^{-5})
3rd	30.0	4.8×10^7	6.1×10^{-6}	3.9×10^{-2}	181	1.4×10^{-3}	4.7×10^{-3}	1.0×10^{-5}
	(25.9)	(6.9×10^7)	(7.8×10^{-6})	(3.5×10^{-2})	(283)	(2.1×10^{-3})	(7.5×10^{-3})	(1.3×10^{-5})
4th	5.3×10^3	1.2×10^7	2.1×10^{-4}	3.5×10^{-2}	111	1.7×10^{-3}	5.2×10^{-4}	1.1×10^{-5}
	(6.4×10^3)	(3.4×10^7)	(4.0×10^{-4})	(7.0×10^{-2})	(118)	(3.4×10^{-4})	(1.0×10^{-3})	(1.2×10^{-5})
Total	6.1×10^2	1.8×10^7	5.8×10^{-5}	3.1×10^{-2}	128	8.4×10^{-4}	2.1×10^{-3}	1.7×10^{-5}
	(2.0×10^3)	(3.6×10^7)	(2.0×10^{-4})	(4.5×10^{-2})	(157)	(1.9×10^{-3})	(4.6×10^{-3})	(1.7×10^{-5})
Coefficient of correlation:[b]								
Wastewater		−0.05	0.99	−0.21	−0.23	−0.14	−0.13	−0.28
Air					0.36	−0.28	0.50	0.24

[a] Mean ± standard deviation (in parentheses).

[b] Coefficients of correlation (r) between enterovirus wastewater or air concentration, as indicated, and other parameters ($n = 16$).

Wilcoxon matched-pairs signed-ranks test (27).

Table 7 contains a list of *Salmonella* serotypes isolated from irrigation wastewater and from sprinklers' airborne emissions. The list of serotypes is ranked according to the frequency of isolates in wastewater. It can be seen that the most common *Salmonella* serotype in the wastewater was *Salmonella anatum*, and that in the air was *Salmonella ohio*. Polioviruses were the most predominant enteroviruses isolated from irrigation wastewater, whereas in the air they were not as common (Table 8).

DISCUSSION

The isolation of low levels of *Salmonella* and enteroviruses from the airborne emissions of sprinkler wastewater irrigation lines demonstrates that these bacteria and viruses can survive natural aerosolization and may be recovered with the procedures used. There is as yet no standard method for recovering viruses from sewage effluents or from airborne emissions, and it is believed that the number of viruses isolated from environmental samples may be one or two logs (90 to 99%) lower than actual concentrations, due to the limitations of the virus recovery procedures. Also, the MPN method used for the enumeration of *Salmonella* spp. is not completely quantitative since it involves a stage of picking colonies from selective media. Despite the limitations of the methods used, it seems that the concentration factors (about 27 m^3 of air into 60 ml of collecting fluid) and the level of sensitivity of the microbial procedures are appropriate for the isolation of airborne pathogenic microorganisms. It may be assumed that higher volume and collecting efficiency will enable better results in sampling low aerosol densities.

TABLE 7. *Salmonella serotypes found in wastewater and airborne emissions*

Serotype	No. of isolates in wastewater	No. of isolates in air
Anatum	6	1
Paratyphi-B	4	0
Hadar	4	1
Emek	4	0
Typhimurium	3	0
Sofia	3	0
Give	3	0
Ohio	2	2
Eastbourne	2	0
Senftenberg	2	0
Typhimurium subsp. *copenhagen*	1	0
Tunis	1	0
Kentucky	1	0
Infantis	1	1
Bredeney	1	0
Montevideo	1	0
Enteritidis	1	0
Incomplete serology	3	0

TABLE 8. *Types of enteroviruses found in wastewater and airborne emissions*

Virus type	No. of isolates in wastewater	No. of isolates in air
Poliovirus[a]	6	0
Type 2	3	1
Type 1	1	0
Echovirus		
Type 1	1	1
Type 7	1	0
Type 11	1	0
Type 21	1	0
Type 24	1	0
Type 29	1	0
Type 25	0	1
Type 17	0	1
Coxsackievirus type B1	0	1
Unidentified	3	2

[a] Poliovirus was identified by the fluorescent-antibody method for the three poliovirus types (see reference 24).

Ten of 18 *Salmonella* serotypes listed in Table 7 are among the 33 serotypes that account for 80 to 90% of the isolates reported from human and nonhuman sources in the United States (19). Two of the five airborne serotypes are listed among these 33 serotypes. The fact that the majority of serotypes isolated from wastewater and air are associated with human infections emphasizes the importance of wastewater airborne emissions in the transmission of salmonellosis.

Table 8 contains a list of virus types isolated from wastewater and airborne emission, ranked according to their frequency in wastewater. Polioviruses were the most commonly found isolates in wastewater, as has been reported previously (9). The frequency of polioviruses in the airborne emissions was lower than that found in the wastewater.

As can be seen from Table 6, the ratios of airborne salmonellae to total coliforms in each quartile and the total mean were higher (26.7-fold in total mean) than the respective ratios in wastewater. The difference was not significant, perhaps due to an insufficient number of samplings. Assuming, however, that the two types of organism had the same opportunity for aerosolization and recovery, this observation indicates that the survival of airborne coliforms was lower than the survival of airborne salmonellae.

Thus coliforms do not fulfill one of the main requirements of a biological indicator, namely, an ability to survive in the environment equal to that of the tested organisms. Low correlation coefficients between *Salmonella* density in wastewater and coliform density in wastewater (0.12), and between airborne *Salmonella* density and airborne coliform density (0.55), indicate that another requirement, namely, a good correlation between the variation in indicator densities and those of the tested organism, is not fulfilled. On the other hand, it must be mentioned that coliforms were present in all air samples tested in these experiments.

It was similarly found (Table 6) that the ability of enteroviruses to survive in the air was also greater than that of coliforms (14.5-fold of the total mean). Correlation coefficients between wastewater densities of enteroviruses and coliforms were low (0.05), as was also found for correlation coefficients between airborne densities of enteroviruses and coliforms (0.36). These findings demonstrate that coliforms do not fulfill two main requirements of biological indicators for enteroviruses.

In all of these experiments, coliforms were present in all air samples tested. If, however, it is assumed from Fig. 1 that the ratio of enteroviruses to coliforms, which increases with the distance, will continue to grow in a linear fashion, an extrapolation may demonstrate a situation in which at a certain distance from the sprinklers (over 100 m), only enteroviruses will still be present in the air. The difference between the mean normalized density of enteroviruses and the mean normalized density of airborne coliforms (×123) indicates a process of specific concentration of airborne enteroviruses in contrast to coliforms. With salmonellae, there is also a difference between their normalized airborne density and that of the coliforms, but it is smaller (5.0-fold).

It has been previously reported that the stability of coliform bacteria, under extreme conditions, appears to be lower than that observed for certain viruses (24) and higher than that for salmonella bacteria (25). Johnson et al. (13) found that the hardiness of enteroviruses in wastewater aerosols was higher than that of both total coliforms and coliphages, which are suggested airborne indicators of human viruses (8).

Our results establish that enteroviruses and *Salmonella* bacteria survive in the air, which is considered a hostile environment for them, better than coliform bacteria. In addition, it appears from our findings that enteroviruses are more hardy than *Salmonella* in wastewater aerosols. Although coliforms were present in all air samples tested, their use as indicators of the microbial contamination of air may result in an inaccurate assessment of the true presence or survival of pathogens.

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